

Applicant : Ken CHIEN
Serial No. : 09/954,571
Filed : September 11, 2001
Page : 3

Attorney's Docket No.: 22000-20660.00/ UC 94-161-9

Amendment to the Claims:

Please amend the claims as follows:

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for delivering an a therapeutic dose of a gene expression cassette in a fluid to cardiac muscle heart for sustained expression comprising steps of:

- (a) providing a viral vector comprising the expression cassette;
- (b) [(a)] increasing dwell time of fluid in a targeted area by induction of inducing complete or near-complete transient cardiac arrest or reversible bradycardia in the cardiac muscle;
- (c) [(b)] administering administration of a vascular permeabilizing permeabilizing agent to the cardiac muscle; and
- (d) [(c)] administering the administration of a viral vector to the cardiac muscle containing a gene expression cassette of interest.

Claim 2 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the dwell time is further comprising increased by the induction of hypothermia in the cardiac muscle.

Claim 3 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the dwell time is further comprising increased by isolation of the heart cardiac muscle from systemic circulation.

Claim 4 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the dwell time is further comprising increased by induction of hypothermia in the cardiac muscle and isolation of the heart cardiac muscle from systemic circulation.

Claim 5 (canceled)

sd-205593

Applicant : Ken CHIEN
Serial No. : 09/954,571
Filed : September 11, 2001
Page : 4

Attorney's Docket No.: 22000-20660.00/ UC 94-161-9

Claim 6 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the dwell time is further comprising increased by induction of reversible bradycardia.

Claim 7 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the vascular permeabilizing agent [[is]] comprises histamine, substance P or serotonin.

Claim 8 (currently amended): The [[A]] method [[as in]] of claim 1, wherein at least one bolus of viral vector virus is administered to the cardiac muscle.

Claim 9 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the viral vector [[is]] comprises an adenoviral vector.

Claim 10 (currently amended): The [[A]] method [[as in]] of claim 9, wherein the adenoviral vector contains comprises a strong cardiac specific promoter.

Claim 11 (currently amended): The [[A]] method [[as in]] of claim [[10]] 9, wherein the adenoviral vector comprises strong promoter contains a cytomegalovirus (CMV) promoter.

Claim 12 (currently amended): The [[A]] method [[as in]] of claim [[10]] 9, wherein the adenoviral vector comprises strong promoter is a Rous sarcoma virus (RSV) promoter.

Claim 13 (currently amended): The [[A]] method [[as in]] of claim 9, wherein the adenoviral vector contains comprises an enhancer elements.

Claim 14 (currently amended): The [[A]] method [[as in]] of claim 13, wherein the enhancer [[is]] comprises a cytomegalovirus (CMV) enhancer.

sd-205593

Applicant : Ken CHIEN
Serial No. : 09/954,571
Filed : September 11, 2001
Page : 5

Attorney's Docket No.: 22000-20660.00/ UC 94-161-9

Claim 15 (currently amended): The [[A]] method [[as in]] of claim 13, wherein the enhancer [[is]] comprises a Rous sarcoma virus (RSV) enhancer.

Claim 16 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the viral vector [[is]] comprises an adenovirus-associated viral (AAV) vector.

Claim 17 (currently amended): The [[A]] method [[as in]] of claim 16, wherein the AAV vector contains comprises a strong cardiac specific promoter.

Claim 18 (currently amended): The [[A]] method [[as in]] of claim 16 [[17]], wherein the adenovirus-associated viral (AAV) vector comprises strong promoter is a cytomegalovirus (CMV) promoter.

Claim 19 (currently amended): The [[A]] method [[as in]] of claim 16, wherein the adenoviral vector comprises strong promoter is a Rous sarcoma virus (RSV) promoter.

Claim 20 (currently amended): The [[A]] method [[as in]] of claim [[9]] 16, wherein the adenovirus-associated viral (AAV) [[AAV]] vector contains comprises an enhancer elements.

Claim 21 (currently amended): The [[A]] method [[as in]] of claim 20, wherein the enhancer [[is]] comprises a cytomegalovirus (CMV) enhancer.

Claim 22 (currently amended): The [[A]] method [[as in]] of claim 20, wherein the enhancer [[is]] comprises a Rous sarcoma virus (RSV) enhancer.

Claims 23 to 30 (canceled)

Claim 31 (currently amended): The [[A]] method [[as in]] of claim 1, wherein the gene expression cassette comprises of interest is a gene fragment.

sd-205593

Applicant : Ken CHIEN
Serial No. : 09/954,571
Filed : September 11, 2001
Page : 6

Attorney's Docket No.: 22000-20660.00/ UC 94-161-9

Claims 32 to 40 (canceled)

Claim 41 (new): The method of claim 1, wherein the viral vector is in a fluid.

Claim 42 (new): The method of claim 9, wherein the adenoviral vector is a replication deficient adenoviral vector.

Claim 43 (new): The method of claim 16, wherein the adenovirus-associated viral (AAV) vector is a replication deficient adenovirus-associated viral (AAV) vector.

Claim 44 (new): The method of claim 1, wherein the vascular permeabilizing agent and the viral vector, or, the vascular permeabilizing agent or the viral vector, are administered by myocardial perfusion.

Claim 45 (new): The method of claim 44, wherein vascular permeabilizing agent or the viral vector is administered before or during, or, before and during, the myocardial perfusion.

Claim 46 (new): A method for delivering a therapeutic dose of a gene expression cassette in a fluid to a heart comprising steps of:

- (a) induction of complete or near-complete transient cardiac arrest or reversible bradycardia in the heart; and
- (b) administration of a vascular permeabilizing agent and a viral vector comprising the gene expression cassette.

Claim 47 (new): A method for delivering a nucleic acid to a heart comprising steps of:

- (a) providing a viral vector comprising the nucleic acid;

sd-205593

Applicant : Ken CHIEN
Serial No. : 09/954,571
Filed : September 11, 2001
Page : 7

Attorney's Docket No.: 22000-20660.00/ UC 94-161-9

(b) inducing complete or near-complete transient cardiac arrest or reversible bradycardia in the heart;

(c) administering a vascular permeabilizing agent to the heart; and

(d) administering the viral vector to the heart.

Claim 48 (new): A method for delivering a nucleic acid to a heart comprising steps of:

(a) providing a viral vector comprising the nucleic acid;

(b) inducing complete or near-complete transient cardiac arrest or reversible bradycardia in the heart; and

(c) administering a vascular permeabilizing agent and the viral vector to the heart.

Claim 49 (new): The method of claim 47 or claim 48, wherein the vascular permeabilizing agent and the viral vector, or, the vascular permeabilizing agent or the viral vector, are administered by myocardial perfusion.

Claim 50 (new): The method of claim 49, wherein vascular permeabilizing agent is administered before or during, or, before and during, the myocardial perfusion.

Claim 51 (new): The method of claim 49, wherein the vascular permeabilizing agent comprises histamine, substance P or serotonin.

Claim 52 (new): The method of claim 1, wherein the expression cassette comprises a mutated form of a gene.

Claim 53 (new): The method of claim 52, wherein the mutated gene is a mutated phospholamban (PLB) that enhances sarco-endoplasmic reticulum calcium ATPase (SERCA-2) activity.

sd-205593

Applicant : Ken CHIEN
Serial No. : 09/954,571
Filed : September 11, 2001
Page : 8

Attorney's Docket No.: 22000-20660.00/ UC 94-161-9

Claim 54 (new): The method of claim 53, whercin the mutated gene is a dominant negative form of phospholamban (PLB).

Claim 55 (new): The method of claim 52, wherein the mutated gene is a dominant negative form of phospholamban (PLB) comprising a mutation at amino acid 16 from serine (S) to glutamic acid (E).

Claim 56 (new): The method of claim 47 or claim 48, wherein the nucleic acid comprises a mutated form of a gene.

Claim 57 (new): The method of claim 54, wherein the mutated gene is a dominant negative form of phospholamban (PLB).

Claim 58 (new): The method of claim 55, wherein the mutated gene is a dominant negative form of phospholamban (PLB) comprising a mutation at amino acid 16 from serine (S) to glutamic acid (E).

Claim 59 (new): A method for delivering a nucleic acid to a heart comprising steps of:

- (a) providing a viral vector comprising the nucleic acid;
- (b) inducing complete or near-complete transient cardiac arrest or reversible bradycardia and hypothermia in the heart;
- (c) administering a vascular permeabilizing agent to the heart; and
- (d) administering the viral vector to the heart.

Claim 60 (new): A method for delivering a nucleic acid to a heart comprising steps of:

- (a) providing a viral vector comprising the nucleic acid;
- (b) inducing complete or near-complete transient cardiac arrest or reversible bradycardia and hypothermia in the heart; and

sd-205593

Applicant : Ken CHIEN
Serial No. : 09/954,571
Filed : September 11, 2001
Page : 9

Attorney's Docket No.: 22000-20660.00/ UC 94-161-9

(c) administering a vascular permeabilizing agent and the viral vector to the heart.

Claim 61 (new): The method of claim 1, wherein the expression cassette comprises a gene regulating cardiac function.

Claim 62 (new): The method of claim 1, wherein the expression cassette comprises a gene for treating cardiac disease.

Claim 63 (new): The method of claim 1, wherein the expression cassette comprises a gene for treating heart failure.

Claim 64 (new): The method of claim 1, wherein the expression cassette comprises a gene regulating cardiac contractility and relaxation.

Claim 65 (new): The method of claim 1, wherein the expression cassette comprises a gene regulating calcium handling in cardiomyocytes.

Claim 66 (new): The method of claim 1, wherein the expression cassette comprises a gene regulating calcium uptake into sarco-endoplasmic reticulum in cardiac cells.

Claim 67 (new): The method of claim 1, wherein the expression cassette comprises a gene encoding sarco-endoplasmic reticulum calcium ATPase (SERCA-2).

Claim 68 (new): The method of claim 1, wherein the expression cassette comprises a gene encoding a polypeptide binding to sarco-endoplasmic reticulum calcium ATPase (SERCA-2) in cardiac cells.

Claim 69 (new): The method of claim 1, wherein the expression cassette comprises a gene encoding a polypeptide that regulates sarco-endoplasmic reticulum calcium ATPase (SERCA-2) in cardiac cells.

sd-205593